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LACK OF MITOCHONDRIAL DNA DIVERSITY IN INVASIVE APPLE SNAILS (AMPULLARIIDAE) IN HAWAII

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ABSTRACT

Three species of apple snails (Ampullariidae) have been introduced to Hawaii. In order to clarify their identities, determine their geographic origins, and evaluate their mtDNA diversity in Hawaii, we sequenced the COI and ND6 mtDNA markers from 103 snails collected on the six main Hawaiian islands. Our samples included *Pila conica* and *Pomacea canaliculata*, whose identities were confirmed by phylogenetic analysis that included other ampullariid species. The third species, *Pomacea diffusa*, known to have been present in the past, was not found. Neither species exhibited any variation at either marker. Both species may have been introduced to Hawaii as single introductions, possibly from the Philippines.

Key words: *Pomacea*, *Pila*, mtDNA, invasive species.

INTRODUCTION

Invasive species are a major concern because of their potential for great ecological, agricultural, human health, and economic impacts (Mack et al., 2000). These alien species may affect native species through predation, competition, introduction of disease, and hybridization (OTA, 1993). Well-known examples include the zebra mussel (*Dreissena polymorpha*), introduced to North America and one of the main factors endangering native freshwater unionid clams, and predatory snails (*Euglandina rosea* and others) introduced to Pacific and Indian Ocean islands, where they have caused the decline and extinction of native snails (Lydeard et al., 2004).

The Ampullariidae include a number of complexes of more or less cryptic species. There are nine recognized genera, the largest of which, *Pomacea* Perry, 1810, has 117 nomenclaturally valid species (Cowie & Thiengo, 2003). The real number of species may be closer to 50, as many synonyms have probably been generated based on minor variations in shell characteristics that may be phenotypically plastic (Estebenet & Martín, 2003). These freshwater snails are known as "apple snails" because of the large, round and sometimes greenish appearance of the shells in some genera, including *Pomacea* (Cowie & Thiengo, 2003). Their biology, including ecology, life-history and behavior, have been reviewed by Cowie (2002).

Native South American apple snails in the genus *Pomacea* were introduced to Taiwan in 1980 with the intention of developing them as a human food resource, both locally and for the international gourmet trade (Mochida, 1991). They were later introduced to other parts of Asia, New Guinea, Guam, and Hawaii, but their development as a food resource was only partially successful, and the snails either escaped or were released and have become major agricultural pests, notably in rice and taro, as well as other crops (Mochida, 1991; Cowie, 2002; Lai et al., 2005; Joshi & Sebastian, 2006). Some species are also common in the aquarium trade, including in Hawaii. In Hawaii, apple snails are now found in the wild predominantly, but not exclusively, in taro-growing areas (Cowie, 1995, 1996; Cowie et al., 2007), where they cause major crop damage (Levin et al., 2006). The environmental consequences of the snails becoming established in Hawaii have not been evaluated, but their voracious and generalist feeding habits (Lach et al., 2001), combined with past experience with other introduced species in the Hawaiian Islands (Staples & Cowie, 2001) and their known eco-

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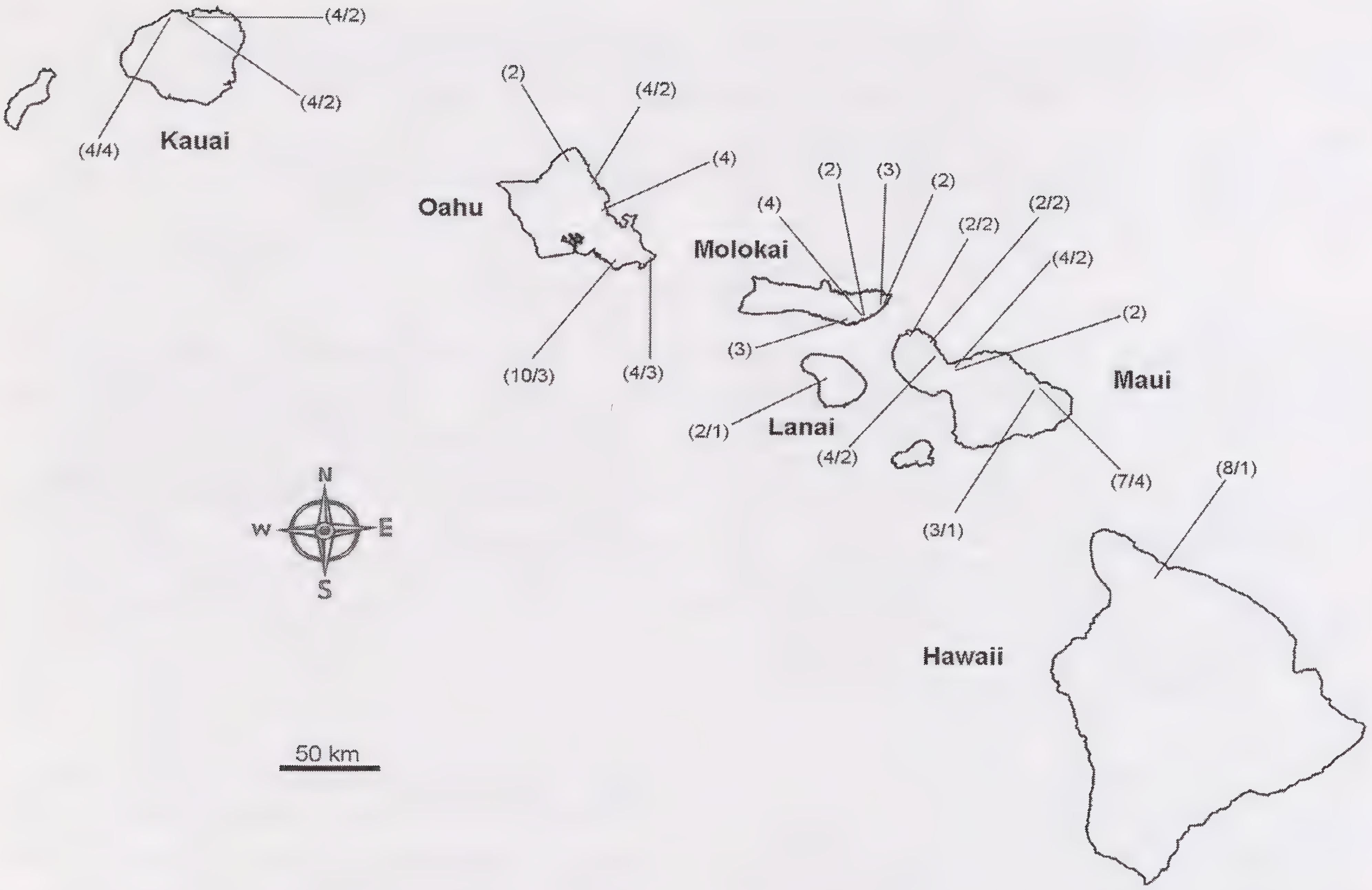

FIG. 1. The Hawaiian Islands showing locations of sampling sites of the 2004–2005 survey from which snails were sequenced (*Pila conica* only on Molokai; *Pomacea canaliculata* on all islands except Molokai). Numbers in parentheses are the number of COI sequences followed by the number of ND6 sequences from each site; single numbers are from sites from which only COI was sequenced; there were no sites from which ND6 only was sequenced.

logical impacts elsewhere (Carlsson et al., 2004), suggest that major damage, including destruction of native vegetation and competition with native freshwater fauna is possible.

Because of their potential negative impacts, it is essential to identify introduced species reliably (Holland et al., 2004). Morphological characteristics are often adequate for species identification, but molecular data are necessary to identify cryptic species that cannot be distinguished using morphological characteristics alone. This is especially the case for *Pomacea*, because the overall highly conserved external morphology across the genus yet considerable intraspecific shell variation obscures the true number of species and their identities (Cazzaniga, 2002). In addition, knowledge of their geographic origins and of their phylogeography in the introduced environment are also potentially important in understanding and managing these invasive snails. Molecular data can more readily address such questions. By sequencing the cytochrome *c* oxidase subunit I (COI) and NADH dehydrogenase subunit 6 (ND6) mitochondrial DNA markers this study aimed to confirm the identities of the introduced ampullariids in Hawaii, determine their probable origin, and investigate any geographic genetic structuring of the introduced populations.

MATERIALS AND METHODS

As part of a survey of apple snail distribution in the Hawaiian Islands during 2004–2005 (Cowie et al., 2007) apple snails were collected from 21 sites on six of the main Hawaiian Islands. Details of these sites and collections are given by Cowie et al. (2007). Five additional collections were obtained from pet stores, an Asian food market in Honolulu, and from other collectors who gave Honolulu as the locality (Fig. 1). All snails were processed and total DNA extracted following Hayes et al. (in press). Amplification of a 658 bp portion of the mitochondrial DNA (mtDNA) encoded cytochrome *c* oxidase subunit I (COI) gene and a 607 bp fragment of the NADH dehydrogenase subunit 6 (ND6) gene was carried out following stan-

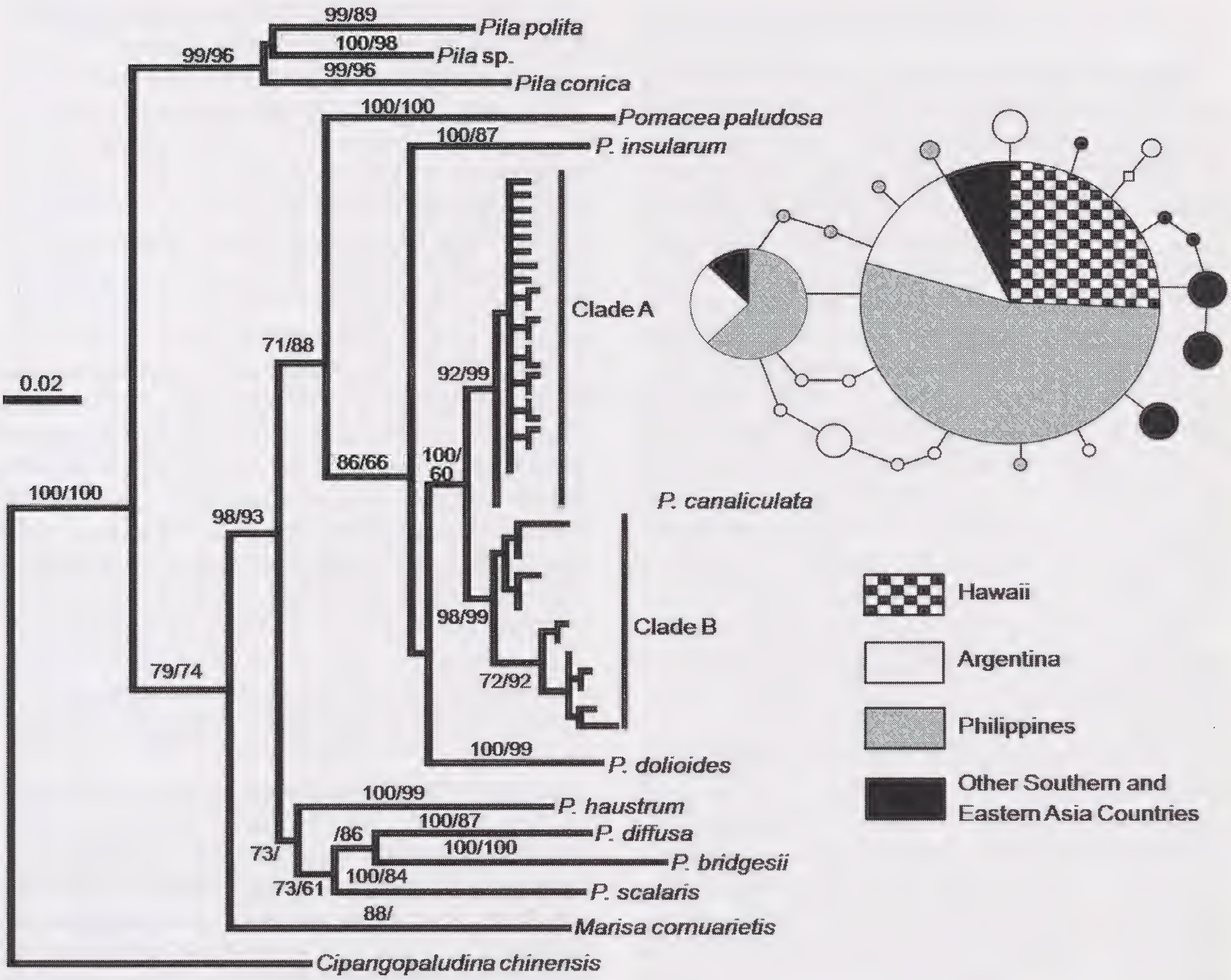

FIG. 2. A COI mtDNA minimum evolution tree and a haplotype network (95% parsimony limit). The phylogenetic tree illustrates the relationships of the two Hawaiian apple snail species, *Pomacea canaliculata* (Clades A and B) and *Pila conica*, to other ampullariid taxa. Terminal branches for all species, except *P. canaliculata*, have been collapsed in order to simplify the tree presentation. Node values represent 500 bootstrap replicates (> 50%) under ME/MP. The haplotype network illustrates the genealogical relationships among *P. canaliculata* individuals in Clade A, the only clade containing Hawaiian samples. The Clade B network (not shown) is composed of haplotypes found in the Philippines, Argentina and a number other Asian countries, the closest one of which is an additional 22 steps from the closest haplotype in Clade A.

dard polymerase chain reaction protocols and primers from Folmer et al. (1994) and Hayes (in prep). Cycle-sequencing, electrophoresis and analyses were carried out on an ABI 3730XL, and all unique Hawaiian sequences generated in this study have been deposited in Genbank (Accession nos. EU523129–EU523131). To definitively identify the Hawaiian snails, all COI sequences were combined with a subset of COI sequences from southern and eastern Asia, the U.S.A., and South America (Hayes et al., in press), and phylogenetic analysis was carried out under minimum evolution (ME) and maximum parsimony (MP) search criteria in PAUP (Swofford, 2003) with 500 bootstrap replicates. Distances for minimum evolution searches were determined using the best fit maximum likelihood model of substitution in MODELTEST (Posada & Crandall, 1998). The ND6 sequences were also compared in a phylogenetic framework to one another and to other samples from the Philippines and the native range in Argentina. Also, COI haplotypes were used to construct a parsimony network with a 95% parsimony limit in TCS in order to illustrate the relationships among the Hawaiian samples and those from other regions. The lack of variation within apple snail species in Hawaii (see Results) prevented further population level analyses.

RESULTS

Based on their morphology and by reference to previous published records of ampullariids in Hawaii (Cowie, 1995, 1996, 1997; Cowie et al., 2007), two species were found: *Pila conica* (Gray, 1828), which is native to south-east Asia, and *Pomacea canaliculata* (Lamarck, 1822), native to South America. The former was found only on Molokai, the latter on all islands except Molokai. The specific identity of the latter was confirmed by phylogentic comparison with COI sequences from material collected from other locations, as stated above (Fig. 2; Rawlings et al., 2007; Hayes et al., in press). A third species, *Pomacea diffusa* (Blume, 1957), previously known from Hawaii although incorrectly identified as *Pomacea bridgesii* (Reeve, 1856), was not found.

We sequenced COI from 14 *Pila conica* and 89 *Pomacea canaliculata*, representing every site, and ND6 from 33 *P. canaliculata*, representing 14 of the 21 sites at which it was found (Fig. 1). All individuals of *Pomacea canaliculata* shared a single COI haplotype and all *Pila conica* also shared a single (different) COI haplotype. The *Pomacea canaliculata* also all exhibited only a single ND6 haplotype, also shared with samples from the Philippines. All analyses yielded congruent phylogenetic trees with only minor differences in support values for deeper nodes (Fig. 2).

DISCUSSION

Pomacea canaliculata was first recorded in Hawaii in 1989, from the island of Maui and subsequently on all main islands except Molokai (Cowie, 1995, 1996), although it may have been present before this (Levin et al., 2007). These snails are popular as a food item among the Filipino community in Hawaii, and there are consistent and extensive anecdotal reports that the Philippines were the source of the introductions to Hawaii (Levin et al., 2007). The single COI haplotype shared by all 89 *Pomacea canaliculata* is also shared by some individuals from Argentina, part of its native range, and is the most common haplotype for this snail in the Philippines (Fig. 2; Hayes et al., in press). The same is true for ND6. Our results are therefore consistent with the explanation that *P. canaliculata* was brought to Hawaii from the Philippines, and for the same reason it was initially taken to Asia, as a human food resource. The *P. canaliculata* from pet stores in Hawaii also share the same COI haplotype and therefore probably originated locally from one of the previously introduced populations. Although this is the most common and widespread haplotype in the Philippines, there are numerous other haplotypes there. The presence of only this one haplotype in Hawaii therefore suggests a single introduction.

Pila conica, was first recorded also from Maui, in 1966, and in 1991 from Oahu and Molokai (Cowie, 1995). During our survey we only found it on Molokai (Cowie et al., 2007), from which *P. canaliculata* is absent. All 14 snails sequenced possessed identical COI haplotypes, indicating that the populations on Molokai may have resulted from a single introduction. This species is also known from the Philippines, where it is native, and it may have been introduced by the same route and for the same purpose as *Pomacea canaliculata*.

An alternative explanation for the lack of mitochondrial genetic variation in the introduced Hawaiian populations could be that strong selection for a single haplotype has led to removal of all others from the populations, a selective sweep. This scenario seems highly unlikely, since it would have had to have taken place on multiple islands and in multiple separate populations within islands, and in two species.

Since ND6 is a faster-evolving gene than COI, particularly in snails (Satler & Steiner, 2004), its lack of variation further supports the interpretation that all individuals of each of the two species are very closely related and that each species may have been introduced just once and from a single population.

The lack of mitochondrial diversity in these introduced populations is surprising, given that *P. canaliculata* especially is an extremely successful invader in Hawaii, and the fact that multiple introductions have occurred in southern and eastern Asia (Hayes et al., in press). Colonization by alien species has often been thought to involve loss of allelic diversity resulting from a genetic bottleneck because of the low numbers of colonists, which in turn may lead to a further reduction in genetic diversity as a result of increased inbreeding. We recorded no diversity at two mtDNA loci, and in principal such a bottleneck would also affect nuclear loci, although to a lesser degree. Such loss of diversity can lead to two consequences. First, population growth is limited and the likelihood of its survival is low (Ellstrand & Elam, 1993). Second, low genetic diversity limits evolution in the population (Sakai et al., 2001). There is often then a lag between initial colonization and proliferation and spread, during which evolution leads to adaptation to the new habitat, development of invasive life-history characters, or purging of the genetic load of inbreeding depression; the genetic constraints are overcome (Mack et al., 2000; Sakai et al., 2001). Although *Pomacea canaliculata* may have been introduced to Hawaii earlier in the 1980s than the first vouchered record indicates, it has not exhibited a significant lag time, but spread rapidly and became a serious pest well within a decade following its introduction (Lach & Cowie, 1999).

Propagule pressure, or the number of separate introductions, is recognized as important in the successful establishment of many introduced species. For example, the European starling (*Sternus vulgaris*) and the house sparrow (*Passer domesticus*) became widely invasive in North America only after repeated introductions (Ehrlich, 1989; Sakai et al., 2001). Multiple introductions may provide the genetic variation needed for many successful invasions (Lavergne & Molofsky, 2007; Roman & Darling, 2007) and may have contributed to the success of *Pomacea* spp. introduced to southern and eastern Asia (Hayes et al., in press). Roman & Darling (2007) have argued that the paradox of the success of invasive species despite low genetic diversity is in fact a “paradox lost”, inasmuch as in many cases multiple introductions led to no loss of genetic diversity, and in those cases exhibiting reduced diversity, their success could be explained in other ways, including the possibility that molecular analyses may underestimate non-neutral genetic diversity, that which would permit success of an invasive species, and that reproductive mode may be important: species able to reproduce asexually might tolerate loss of genetic diversity more readily. This latter may be the case in invasive freshwater clams in the genus *Corbicula*, of which a single clonal lineage extends from Michigan to Patagonia (Lee et al., 2005).

Some instances of reduced diversity can be explained on a case-by-case basis. For instance, introduced Argentine ants (*Linepithema humile*) exhibit much reduced genetic variation, but this is the key to their success as an invasive species (Tsutsui et al., 2000), because reduced variation permits the formation of super-colonies that are more successful than normal colonies because of the reduction in costs associated with territoriality.

However, while we were not able to assess non-neutral genetic diversity, ampullariids are obligately sexual reproducing species with separate sexes. It indeed remains paradoxical, then, that *Pomacea canaliculata* and to a lesser extent *Pila conica* are such successful invaders in Hawaii, despite their extremely reduced mitochondrial variation. Similarly, the success of the invasive freshwater snail *Ferrissia fragilis* in Europe, which exhibits little genetic variation in its introduced range, remains unexplained despite speculation that its biological attributes may be important (Walther et al., 2006). Equally, reasons for the success of the introduced Indo-Pacific fish *Fistularia commersonii* in the Mediterranean despite the presence of only two mtDNA haplotypes are unknown (Golani et al., 2007).

However, it may not be necessary to invoke multiple introductions to account for their success. Preliminary analyses of mitochondrial diversity in the native ranges of various *Pomacea* species show that frequent bottlenecks have played a role in structuring populations (Hayes et al., in press). This may have permitted *Pomacea canaliculata* and other invasive species to become successful despite reduced genetic diversity.

As Roman & Darling (2007) said, “although measurements of relative genetic diversity are becoming more common, research into the mechanisms explaining the success of low-diversity populations lags behind considerably”, contradicting their “paradox lost” assertion. Research to further our understanding of how genetic diversity is related to invasiveness remains crucial in development of management strategies for alien species.

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